ABSORPTION AND DISPOSITION OF THE ANTI-INFLAMMATORY DRUG FLOSULIDE (CGP 28 238) IN ONE HEALTHY MALE VOLUNTEER.

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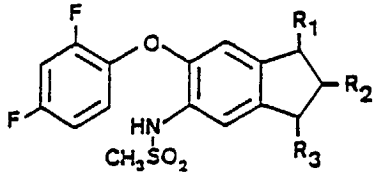
Flosulide (I) is a potent non-steroidal anti-inflammatory drug currently undergoing clinical trials for the treatment of chronic inflammatory diseases such as rheumatoid arthritis and osteoarthritis.
Absorption and disposition of 14C-labelled I were studied in one healthy male volunteer after a single peroral dose of 40 mg. Analysis of drug and metabolites in plasma and urine was performed by HPLC-UV, HPLC-diodearray spectroscopy and HPLC-radiometry. Metabolites were identified based on co-chromatography with synthesized reference compounds.

No.	R1	R2	R3
I	=O	-H	-H
III	=O	-H	-OH
IV	=O	-OH	-H
V	-OH	-OH	-H (cis)
VIII	-OH	-OH	-H (trans)



I was absorbed to at least 58% and accounted for half of the total plasma radioactivity (14C-AUC). I was eliminated from plasma with a half-life of 9 h, and total 14C with a terminal apparent half-life of 38 h. The main plasma metabolites were V (24% of 14C-AUC), III (12%) and IV (6%).
The bulk of the dose (83%) was excreted within 2 days, excretion was complete after 5 days. The renal route dominated. Various dihydroxy- and hydroxy-metabolites were excreted in urine, partly as glucuronic acid conjugates. Quantitatively most important were metabolites V and VIII.

EFFECTS OF MIXED TYPE INDUCERS ON THE ANDROSTENEDIONE METABOLISM IN LIVER MICROSOMES OF Ah-RESPONSIVE AND Ah-NONRESPONSIVE MICE

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As it is known, hydroxylations of androstenedione (AD) in different positions are specific reactions for cytochromes P-450IIB1, IIC11, IIA1 and IIIA1. Treatment of Ah-responsive (CBA) and Ah-nonresponsive (DBA) mice with phenobarbital caused 3-5-fold increase of AD 16β-hydroxylation and 20-fold increase of AD 6β-hydroxylation. It was shown, that antibodies against P-450 IIB1 inhibited 16β-hydroxylation of AD both in mice and rats while the reaction of 6β-hydroxylation was not inhibited by these antibodies. It is P-450 of the IIIA family that is probably involved in this reaction.Treatment of mice with hexachlorobenzene or Aroclor 1254 was accompanied by an increase of 16β-hydroxylation activity in CBA mice only. In microsomes of DBA mice this activity was not changed giving evidence for Ah-receptor dependence of P-450 induction. The same result was obtained for the 16α-hydroxylase activity. 6α-hydroxylase activity was induced in both DBA and CBA mice.
Thus we conclude that the Ah-receptor can be involved in the mechanism of mixed type induction of P-450 IIB1, whereas the P-450 IIIA induction does not depend on this receptor.

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MECHANISM OF THE GSH-DEPENDENT PROTECTION AGAINST MICROSOMAL LIPID PEROXIDATION

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Several explanations have been provided for the GSH-dependent protection against microsomal lipid peroxidation. The involvement of either a phospholipid hydroperoxide GSH-peroxidase (PLHG-px), the microsomal GSH-transferase (GSH-tr) or a free radical reductase has been suggested. In this study the species difference in the ability of GSH to protect against lipid peroxidation in liver microsomes was used to further evaluate the protective mechanism. It was found that 1 mM GSH induced a lag phase in the time course of lipid peroxidation of 35, 15 and 5 minutes in respectively guinea pig, rat and mouse liver microsomes. Using heated or trypsin treated or vitamin E depleted microsomes, in no case a lag phase was induced by 1 mM GSH. Determination of GSH-tr activity in these species showed that there was no correlation between the GSH-tr activity and the extent of the GSH-protection, indicating that the GSH-tr is not responsible for the protection. There was also no correlation between the effect of GSH and the vitamin E content of the microsomes. Since mercaptoethanol was not able to substitute for GSH in the protection, the involvement of PLHG-px does not seem to be very likely, because for this enzyme mercaptoethanol is an equipotent cofactor as GSH. It was found that during the protection against lipid peroxidation by GSH, the consumption of vitamin E by oxidative stress was also prevented. This is in line with the hypothesis that GSH protects via a free radical reductase that recycles vitamin E that is otherwise consumed during oxidative stress. The difference in the extent of the GSH-dependent protection observed in guinea pig, rat and mouse microsomes might be due to a difference in activity of the free radical reductase in these species.

EFFECTS OF THE FUNGICIDE FOLPET ON GLUTATHIONE LEVELS IN THE GASTROINTESTINAL TRACT FOLLOWING ORAL ADMINISTRATION TO MICE AND RATS

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Glutathione (GSH) is involved in the metabolism of folpet, N-(trichloromethylthio)-4-phenyl-1,2-dicaroximide, in the gastrointestinal tract (GI tract) of both mice and rats. Groups of male CD1 mice and Sprague-Dawley rats were administered folpet either (i) in the diet for 21 consecutive days at levels of 0, 50 or 5000 ppm, or (ii) by a single oral gavage dose at levels of 0, 7.6, 72 or 668 mg/kg; a positive control group received diethylmaleate at 600 mg/kg. Animals were killed either (i) at the end of the dietary period, or (ii) at 0, 0.5, 1, 2, 6 or 24 h post-dose. The stomach, duodenum, jejunum, ileum and liver were removed from each animal for determination of GSH concentration. Following 21 days' administration of dietary folpet at 5000 ppm, there were statistically significant increases in GSH levels in the duodenum, jejunum and ileum of both species; the magnitude of this change was consistently greater in mice than in rats. There were also small increases in duodenal and jejunal levels of GSH in animals receiving 50 ppm folpet. Folpet did not appear to affect hepatic GSH levels in the rat, but caused a significant decrease in mice fed 5000 ppm. A single oral gavage dose of diethylmaleate initially depleted GSH in all tissues, with a 'rebound' to above control values in some tissues at 24 h. Folpet also depleted GSH levels, in a dose-dependent manner, in each region of the GI tract at 0.5 h post-dose. A subsequent 'rebound' of GSH levels in GI tract tissues occurred. At 24 h post-dose, animals given 668 mg/kg folpet also showed a reduction in hepatic GSH levels, possibly indicative of a depletion of GSH precursors within the liver. The mouse appeared to be more sensitive than the rat in all these effects. These results indicate that GI tract tissue GSH levels in the mouse respond more than those in the rat to folpet. This is possibly the consequence of a greater reliance by the mouse on GSH for the detoxication of folpet. Coupled with the lower absolute quantity of GSH in the mouse, this observation provides a biochemical basis for the different sensitivities of the two species to the tumorigenic effects of the fungicide.

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PHARMACOKINETICS OF LIDOCAINE AND 2,6-XYLIDINE IN DOGS.

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The metabolism of lidocaine is extensive in both animals and man. The metabolic pathways involved are aromatic hydroxylation, amide hydrolysis and dealkylation. 4-Hydroxy-2,6-xylidine is the major metabolite excreted in the urine of most species. This metabolite can be formed from a number of metabolic intermediates, such as 4-hydroxy-lidocaine, 4-hydroxymonoethylglycine-xylidine, 4-hydroxyglycinexylidine and 2,6-xylidine. In order to elucidate if aromatic hydroxylation occurs prior to amide hydrolysis, we conducted a study where we simultaneously injected equimolar amounts of lidocaine and [$^2H_3$]-2,6-xylidine to dogs followed by serial blood sampling during a 24 h period. The amount of lidocaine, 2,6-xylidine, [$^2H_3$]-2,6-xylidine in the plasma were analyzed by HPLC and GC-EIMS, and the amount of labeled and unlabeled 4-hydroxy-2,6-xylidine in urine was quantified by LC-MS. The ratio of the areas under the plasma concentration versus time curve for labeled and unlabeled 2,6-xylidine suggests that the fraction of the lidocaine dose metabolized to 2,6-xylidine in dogs was around 26%. The findings imply that direct hydrolysis of the amide bond is an important metabolic pathway of lidocaine in dogs.

BUFURALOL HYDROXYLATION IN NON-HUMAN PRIMATE LIVER MICROSOMES AS A MODEL OF HUMAN CYTOCHROME P450IID6

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Deficiency in drug oxidation of the debrisoquine/sparteine type polymorphism affect more than 25 drugs used in clinical practice. This deficiency leads to considerable variations in the pharmacokinetics and toxicology in patients recieving these drugs, and consequently there is a need for a rapid and reliable model to screen new chemical entities for interaction with the human cytochrome P450IID6 (P450db1, P450buf1) before giving the drug to humans. Until today, no animal model has been described as a working model of this drug oxidation deficiency in man.
However, using human liver microsomes and substrates like debrisoquine, sparteine, dextromethorphan and bufuralol, one can easily detect potential new substrates of P450IID6 using simple in vitro inhibition assays, i.e. look for inhibition of the formation of polymorphic metabolites. Nevertheless, human liver samples are not easy to obtain, and we undertook a study to look at the non-human primate African Green monkey (Cercopithecus aethiops) as a working model of the human debrisoquine/sparteine drug oxidation polymorphism.

(+)-bufuralol was hydroxylated in liver microsomes of C. aethiops by a NADPH/O_2 dependent reaction. Most livers showed biphasic kinetics indicating that at least two enzymes are involved. The mean K_m values were 20.7 and 412 µM for the high and low affinity enzyme, respectively, and the corresponding mean V_{max} values 30 and 45 nmol/mg/60 min, respectively. The reaction was inhibited by quinidine and quinine, with K_i-values of 0.4 and 4.0 µM, respectively. (-)-bufuralol 1'-hydroxylation showed similar biphasic kinetics in the same primate livers with mean K_m values of 20.0 and 180.2 µM, respectively, and mean V_{max} values 3.6 and 10.7 nmol/mg/60 min.
The stereoselectivity of bufuralol-hydroxylation and inhibition by quinidine and quinine is similar to man and indicate that African Green monkey could be used as a rapid screening model with regard to interaction of new substances with human cytochrome P450IID6. The model is currently beeing validated using other known inhibitors of P450IID6 and immunodetection of enzyme protein with specific monoclonal antibodies raised against purified human liver enzyme (P450buf1).

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PROBING THE ACTIVE SITE OF HUMAN CYTOCHROME P450IID6 USING 1,4-DISUBSTITUTED PIPERAZINE AND PIPERIDINE DERIVATIVES.

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Defiency in drug oxidation by the debrisoquine/sparteine-type polymorphism affects numerous drugs. Today, more than 25 drugs representing β-adrenergic blocking agents, antiarrhytmics, antidepressants and opoids have been reported as sustrates of human cytochrome P450-IID6 [1]. These compounds represent a vast range of chemical structures with the only common characteristic of (at least) one basic nitrogen atom.GBR 12909 (1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)-piperazine; Fig. 1) was recently reported to be a potent inhibitor of sparteine oxidation in human liver microsomes [2]. Based on these findings the structure-activity relationships for the inhibition of bufuralol 1-hydroxylation was investigated with a number of derivatives of GBR 12909. Two major conclusions emerged from the study: 1) the size of the 1-substituent has no effect on the inhibitory effect of the compounds and 2) the presence of a terminal phenyl group at the 4-position was favorable for the inhibitory potency of the piperazine and piperidines studied. Further, the involvement of P450-IID6 in the metabolism of the derivatives was investigated with a predictive model for substrates of the P450-IID6 [3]. The compounds predicted to be metabolized by P450-IID6 in effect were potent inhibitors of bufuralol hydroxylation. Interestingly, the computer model predicted in addition that the non-planar biphenyl substituents at the 1-nitrogen atom could not be accomodated into the active site. This was in good agreement with the finding that the 1-substituent was not involved in the inhibition of P450-IID6. Replacement of the 4-nitrogen atom by a carbon atom, i.e. changing piperazine derivates into piperidine analogs, had no influence on the inhibitory potency of the compounds. This observation was further supported with simple amine like 4-benzylpiperidine, 1-phenylethylpiperidine and 4-benzylpiperazine (K_i values towards bufuralol hydroxylation 5, 20 and 40 μM, respectively). These results indicate that 4-substituted (aromatic) piperidines are general structures for inhibition of cytochrome P450-IID6 which is in good agreement with the potent inhibition of P450-IID6 by the neurotoxin MPTP and the serotonin re-uptake inhibitor paroxetine (both containing structural elements closely related to the 4-benzyl piperidine structure).

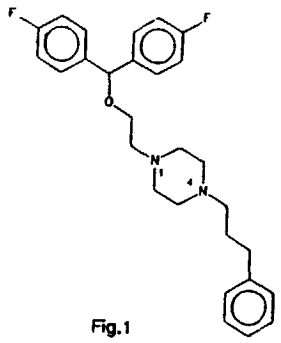

Fig.1

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PLASMA LEVELS OF REMOXIPRIDE OVER A 24-HOUR PERIOD FOLLOWING DIETARY ADMINISTRATION IN RAT

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The concentrations of a drug in plasma and in other tissues following repeated administration, are important factors to be considered in the toxicological evaluation of the drug. Dietary administration is a common route of exposure in long-term toxicity and carcinogenicity studies. Since rats are nocturnal and feed primarily at night, blood sampling on one single occasion during a 24 hour dosing period via diet does not represent the plasma levels during the entire period of dosing. The choice of a single time point for blood sampling is therfore suboptimal. The objective of the present study was to determine the plasma concentrations of remoxipride (RMX), a selctive dopamine D2 receptor antagonist, during a 24 h long period following dietary administration. Male and female Sprague-Dawley rats were subjected to a 6 week regimen of oral remoxipride given as an admixture in the diet at the dose levels of 45 and 145 μmol/kg/day. At the end of the test period and at 3-hourly intervals over the ensuing 24 h period the blood was collected. A clear circadian varation in plasma concentrations of RMX was observed in rats of both sexes receiving the high dose but this was less marked in animals given the low dose. In the high dose groups the RMX concentrations in liver, lung and kidney were considerably higher than in plasma (10-100-fold) whereas in the low dose groups the plasma and organ levels were comparable. Our results show that the time of sampling in long-term studies is important and that a number of target tissues are exposed to considerable concentrations of RMX also during the period when the the plasma levels are low.

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REMOXIPRIDE IS A POOR INDUCER OF HEPATIC P450s IN RAT.

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Remoxipride (RMX), a substituted benzamide, is a selective dopamine-D2 receptor antagonist and clinical trials have shown that the compound is effective in the treatment of acute schizophrenia. In order to assess possible induction of hepatic microsomal enzymes by RMX male Sprague-Dawley rats were treated daily for 8 days with RMX orally by gavage at doses of 20 and 100 μmol/kg. Liver weight (as percent of body weight) and total cytochrome P450 content were not affected by either dose of RMX. Western immonoblot analysis of microsomes using polyclonal antibodies (OXYgene) raised against liver P450IA1, P450IIB1 and P450IIIA1 revealed no increase in the amount of the hemoproteins investigated, following RMX-treatment at the high dose level. Liver microsomes from rats pretreated with RMX showed slightly increased rates of O-demethylation of p-nitroanisole, N-demethylation of aminopyrine and aromatic hydroxylation of aniline as compared to control microsomes at both dose levels. The rate of disappearance of RMX in liver microsomes from RMX-pretreated rats were lower than that in microsomes from control rats. In summary, although RMX is a poor inducer of rat hepatic P450s, RMX and/or its metabolites seem to interfere with its own metabolism on the level of microsomal enzyme systems following subchronic treatment in rat.

PARACETAMOL CYTOTOXICITY AND INTRACELLULAR CALCIUM IN ISOLATED HAMSTER HEPATOCYTES

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It has been proposed that paracetamol exerts its toxicity by a mechanism involving an early and sustained elevation of cytosolic calcium. The aim of the present study was to evaluate this proposal by direct measurement, using the fluorescent probe fura-2, of intracellular calcium in paracetamol toxicity to hamster hepatocytes.

Incubation of the cells with 1 mM paracetamol for 30 min (Phase I) resulted in a marked loss of viability (50 % ; as assessed by trypan-blue exclusion) over a further 210 min of incubation (Phase II) in fresh medium, without paracetamol. Incubation with paracetamol did not change cytosolic calcium concentrations during Phase I. In Phase II loss of viability was accompanied by an increase in intracellular calcium from 190 $\pm$ 37.9 nM to 308 $\pm$ 49 nM (mean $\pm$ S. D. n=5.) It was not possible to separate these two events. Incubation with 5 mM paracetamol resulted in a greater loss of viability (to only 9 % over 210 min) and greater increases in cytosolic calcium. However, calcium elevations did not precede loss of viability. Incubation of hepatocytes in calcium- free medium did not ameliorate cell-death observed with 1 mM paracetamol. In addition, prior depletion of intracellular calcium stores with vasopressin (100 nM; 30 min) did not protect against the toxicity of 1 mM paracetamol.

These results provide evidence that, under the conditions of these experiments, elevations in cytosolic calcium accompany loss of viability but do not precede it. There is no early sustained rise in cytosolic calcium following incubation with paracetamol. Thus, the role of raised intracellular calcium in the initiation of paracetamol cytotoxicity is not supported by these studies.

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PREDICTION OF PHENYTOIN CLEARANCE FROM HEPATOCYTE DATA. EFFECT OF HEPATOCYTE INCUBATION MEDIA.

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Phenytoin (P) is a useful model compound for establishing *in vitro - in vivo* relationships. It is essentially completely metabolised via a single oxidative pathway. Its metabolic clearance is low and readily saturated.

We have characterised P disposition *in vivo* in a group of 30 rats with previously implanted cannulae by means of intravenous infusions. A specific radiometric assay demonstrated a range of steady state plasma P concentrations of 1.6-90μM which could be described by a modified Michaelis-Menten model. A Vmax of 93 nmoles/min/250g B. wt. and a Km of 11μM described a high affinity, low capacity site whereas a second low affinity site gave a clearance of 1.2ml/min/250g B. wt.

P metabolism by hepatocytes could also be described by a two site Michaelis-Menten model; the relative importance of the two sites being similar to *in vivo*. The clearance associated with the high affinity site was highly dependant on the media used for hepatocyte incubation. When compared to a standard phosphate buffered saline media, the presence of newborn calf serum, bovine serum albumin or Williams medium E influenced P oxidation rates. Hence not all extrapolated clearances from hepatocytes were consistent with *in vivo* findings.

SEPARATION OF ACIDIC METABOLITES OF 3-CHLORO-4-(DICHLOROMETHYL)-5-HYDROXY-2(5H)-FURANONE (MX) BY HPLC

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The potent drinking water mutagen, MX, which has been shown to be formed following chlorination of humic waters, was administered perorally to rats (20 mg/kg). The urine samples were analyzed for ^{14}C-radioactivity using a liquid scintillation counting. No unmetabolized MX was found in the urine when analyzed by HPLC and radioactivity monitoring. The metabolites were highly water-soluble and acidic. For the determination of free forms of conjugated metabolites, concentrated urine samples were incubated with ß-glucuronidase, with sulfatase or with HCl. The preliminary results suggest that there are neither glucuronide nor sulfate conjugates of MX in urine. At least seven as far unidentified metabolites of MX have been detected.

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METABOLISM OF 2-ETHYLHEXANOIC ACID IN THE RAT IN VIVO

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The metabolism of 2-ethylhexanoic acid (2-EHA), a possibly toxic biotransformation product of the widely-used plasticizer di(2-ethylhexyl)phtalate (DEHP), was studied in rats in vivo. Following ip administration of 400 mg/kg sodium 2-EHA, a total of eleven metabolites were identified in urine by GC/MS techniques and with the aid of authentic compounds prepared by synthesis. The principal pathways of 2-EHA-metabolism were, in addition to ester glucuronide formation, ω-oxidation, (ω-1)-oxidation and β-oxidation. This indicated that both cytochrome P-450 and mitochondrial enzyme systems participated in the oxidative biotransformation of 2-EHA. As shown recently, cytochrome P-450 was also involved in the dehydrogenation of 2-EHA to the terminal olefin 2-ethyl-5-hexenoic acid, the most important unsaturated metabolite found in urine. In view of the toxic potential of terminal olefins, biotransformation of 2-EHA to 2-ethyl-5-hexenoic acid may contribute significantly to the toxic properties of 2-EHA.

GLUCURONIDATION OF 3'-AZIDO-3'-DEOXYTHYMIDINE (AZT) IN HUMAN LIVER MICROSOMES: ENZYME CHARACTERIZATION AND DRUG INTERFERENCES

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Upon administration to man, 3'-azido-3'-deoxythymidine (AZT, Retrovir) is extensively metabolized as 5'-O-glucuronide. The human liver microsomal UDP-glucuronosyltransferase (UDPGT, EC 2.4.1.17) involved in the reaction was monitored by a sensitive HPLC method. AZT glucuronidation proceeded with an apparent Vmax of 0.98 nmol/min/mg protein and Km of 13 mM. The enzyme was selectively induced four-fold by phenobarbital in rat. Gunn rats, genetically deficient for bilirubin UDPGT were still able to glucuronidate AZT. All together the data suggested that phenobarbital-inducible isozymes catalyzed the reaction (1).

Measurement of AZT glucuronidation in various human hepatic samples revealed a large inter-individual variation (27%). Several compounds that have been reported to interfer with AZT metabolism were tested as possible *in vitro* inhibitors of AZT glucuronidation in human liver microsomes. Paracetamol, salicylic acid, clofibric acid, cimetidine, norethindrone, isoniazide, rifampicin, dapsone, trimethoprim, sulfamethoxazole did not affect the glucuronide formation. By contrast probenecid, naproxen, flurbiprofen, ethynylestradiol, lorazepam, codeine, morphine were able to inhibit in a dose dependent fashion. The best inhibitors flurbiprofen, naproxen and probenecid could competitively inhibit AZT glucuronidation with an apparent K_i of 50, 172 and 900 uM, respectively.

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THE METABOLIC CONVERSION OF THE PRO-DRUG LOPERAMIDE OXIDE TO LOPERAMIDE IN VITRO AND IN VIVO.

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Loperamide (LOP) is an effective antidiarrhoeal. In order to keep more drug available for local action in the intestine, thus reducing the systemic absorption of LOP, loperamide oxide (LOX) was synthetized and its pharmacological effect was compared with that of the parent drug. LOX was found to be a pro-drug requiring metabolic conversion to LOP. This was studied *in vitro* and *in vivo* in rats and dogs, using appropriate techniques to measure both LOX and LOP.

LOX was rapidly reduced when incubated *in vitro* with the contents of the small intestine and the caecum. Reduction required anaerobic conditions and the reductase activity gradually increased from the small intestine to the caecum. Anaerobic bacteria, isolated from the gut, played a predominant role in the conversion of LOX to LOP. The role of the microflora was further substantiated in experiments with gut contents from germ-free rats, although it was also demonstrated that factors of non-bacterial origin could mediate reduction. The uptake of LOX by isolated intestinal epithelial cells of dogs and rats was slower and more limited than that of LOP. In these cells, as well as in isolated hepatocytes, reduction of LOX could be demonstrated.

In vivo studies revealed that after oral administration of LOX to the dog, peak plasma levels of metabolically formed LOP were reached later and were on average 50 % lower than after administration of an equal dose of LOP. Simultaneous blood sampling in the portal and hepatic vein in conscious dogs further revealed that LOX was absorbed to a lower extent than LOP and that both drugs were metabolized in the gut. Metabolism studies in dogs, showed that after an oral dose of LOX, 7.5-9 % of the dose was excreted in the faeces as LOP, but less than 2 % after administration of LOP.

In conclusion, the present experiments demonstrate that lower plasma levels of LOP will be obtained and more LOP will be available for its antidiarrhoeal activity in the lower intestinal tract after administration of LOX than after LOP itself. The resulting data thus provide a pharmacokinetic basis for the increased safety profile of LOX.

POLYMORPHIC N-ACETYLTRANSFERASE: A COMPARISON OF PHENOTYPE AND GENOTYPE.

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N-acetyltransferase polymorphism (pNAT) has been associated with several disease susceptibilities in man based on phenotypic studies involving the use of probe drugs (e.g. Isoniazid). An Isoniazid phenotyping method has been compared with the genotype of NAT in a Japanese population using the Southern blotting technique (1). We report a simple method of genotyping pNAT making use of specific point mutations characterising the different pNAT alleles which have been identified within the open reading frame of this gene. PCR is used to amplify specifically the polymorphic NAT alleles. The resulting product is then digested with restriction endonucleases whose cleavage sites are affected by the allele specific point mutations in pNAT. The four NAT alleles whose sequences have been published can be differentiated by this method. NAT genotype determined in this way has been compared with NAT phenotype using Sulphamethazine as the probe drug. Comparison of allele frequency in a European Caucasian population with the previously published Japanese population (1) suggests a basis for the inter-ethnic difference in the NAT polymorphism and may explain the higher proportion of fast acetylator phenotypes in the Japanese population.

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Fe(III)-CONTAINING HEPATOBILIARY CONTRAST AGENTS FOR MAGNETIC RESONANCE IMAGING

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We have examined the biodisposition and hepatobiliary transport of several potential magnetic resonance imaging (MRI) contrast agents, the Fe(III) chelates of desferrioxamine B (DF), N-(glutaryl)desferrioxamine B (GDF), N-(3-phenylglutaryl)desferrioxamine B (PGDF), the methyl ester of PGDF (Me-PGDF), N,N'-bis(2-hydroxybenzyl)ethylenediamine-N,N'-diacetic acid (HBED) and N,N'-ethylenebis(2-hydroxyphenyl-glycine) (EHPG). Urinary and fecal excretion of ^{59}Fe over 7 days in rats given tracer doses were 81±4 and 5±1%, DF; 83±1 and 7±1%, GDF; 44±1 and 34±1%, PGDF; 48±11 and 31±10%, Me-PGDF; 22±1 and 67±2%, HBED; and 26±5 and 22±2%, EHPG. In bile duct-cannulated rats the biliary excretion of ^{59}Fe from ^{59}Fe-chelates of the anions PGDF, HBED and EHPG and the uncharged Me-PGDF was inhibited by saturating doses of the anion bromosulfophthalein (BSP) but not by taurocholate or the cation oxyphenonium. Using the MIDAS computer graphics program we have determined that the common structural features of these chelates which might be recognized by the BSP transporter include an aromatic ring connected to an oxygen atom that can lie to the side of the ring and above its plane. We continue in our efforts to design other agents with optimal kinetic and imaging properties.

METABOLIC DISPOSITION OF ^{14}C-CI-943 IN MAN

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INTRODUCTION

CI-943 is a new compound which is currently under investigation for the treatment of psychotic disorders. Previous studies in rats and monkeys have shown that CI-943 is metabolized extensively. This study was conducted to determine in humans the structure of the metabolites, the mass balance and the metabolite profiles.

METHODS

Six healthy male volunteers received a single oral dose of 200 mg ^{14}C-CI-943 (94 µCi). The radioactivity was measured in blood, plasma, urine, and feces and metabolite profiles were recorded by HPLC with radiometric detection. The metabolites of CI-943 were isolated from both, plasma and urine, by multidimensional HPLC. The structures were determined by chromatographic comparisons to previously identified metabolites, by mass spectrometry and by NMR.

RESULTS AND DISCUSSION

Within 168 hours after dosing, recovery of the radioactivity was 80.0 ± 7.66% in urine and 13.6 ± 6.27% in feces (mean ± SD). The total recovery was 93.6 ± 1.72%. The maximum plasma concentration was 6.18 ± 1.32 µg eq/mL and the maximum blood concentration was 5.48 ± 1.09 µg eq/mL. The metabolite profiles in plasma and urine were similar in that they showed the same major metabolites. CI-943 and seventeen of its metabolites were purified form urine and the structures were elucidated by MS and NMR. Using comparative chromatography and MS, the structures of nine metabolites, purified from plasma, were determined. CI-943 was extensively metabolized in man by different oxidative processes followed by the partial glucuronidation of the resulting hydroxy compounds. The comparison of the biotransformation of CI-943 in man, monkey and rat, determined in earlier studies, shows qualitative and quantitative differences.

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NOVEL DUAL–LABEL TECHNIQUE TO STUDY FELODIPINE METABOLISM IN VITRO

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Felodipine, a 1,4–dihydropyridine type Ca–antagonist used for treatment of hypertension, is a high clearance drug which is entirely eliminated by metabolism both in animals and humans. The primary metabolic step is oxidation of the dihydropyridine to the corresponding pyridine metabolite (M II). In this study, a mixture of [^{14}C]felodipine (10 µM) and tritium labelled M II metabolite (1 µM) was incubated in man, dog, rat liver microsomes and rat liver cells. Frequent samples from the incubation solution were analysed by reversed phase HPLC with flow through, dual label radioactivity detection. Eleven metabolites were quantified by peak integration in both isotope channels and their kinetics were calculated. This experimental approach allowed for

- direct detection of a new primary (only ^{14}C–labelled) dihydropyridine metabolite formed by ester hydrolysis
- comparisons of the relative half–lives of felodipine and the M II metabolite
- estimation of rate limiting steps for M II metabolism in a single experiment.
- kinetic and chemical evidence of a new metabolic sequence.

Of the detected radioactivity, about 75% (^{14}C) and 85% (^{3}H) can be accounted for as identified metabolites. Interspecies variations in metabolic pathways were observed, e.g. the highest capacity for hydroxylation was found in the rat and ester hydrolysis was more rapid in the dog.

INDUCED GLUTATHIONE SYNTHESIS IN PIG HEPATOCYTES EXPOSED TO FURAZOLIDONE

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Monolayer cultures of pig hepatocytes were incubated with the nitrofuran drug furazolidone for 16 h (50-500 µM). Intracellular GSH levels were apparently unaffected, contrasting with the fact that GSH is thought to be involved in both the scavenging of reactive drug intermediates and in the detoxification of hydrogen-peroxide, formed as a result of redox-cycling of the initially formed radical nitro anion. It was hypothesized that this discrepancy might be due to an increased GSH synthesis by the cells, especially considering the high levels of the required amino acid in the incubation medium.

This was confirmed by incubating cells with furazolidone in the presence of 4 mM BSO, an inhibitor of GSH synthesis, showing a clear dose-related decrease in intracellular GSH-levels. In the presence of 40 µM BCNU, an inhibitor of GSH-reductase, GSH-levels were almost doubled at a drug dose of 50 µM and slightly decreased at 500 µM, indicating that the loss of GSH is at least partly due to the formation and excretion of GSSG.

Further proof was obtained by incubating cells, containing ^{35}S-labelled GSH, with furazolidone, resulting in unchanged GSH-levels, but a clear loss of the labelled GSH. In the medium about 40% of this loss was accounted for by increased levels of labelled GSSG, and the other 60% by increased levels of a labelled compound which according to its chromatographic behaviour, could be CysSG, known to be formed after excretion of GSH into the medium. No evidence could be obtained for the formation and excretion of a reactive GSH-conjugate of furazolidone, as shown previously with microsomes.

It is concluded that exposure of pig hepatocytes to furazolidone results in a clear loss of GSH, due to the excretion of GSSG and possibly GSH, but that the loss of GSH is compensated for by an increased GSH-synthesis.

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PERCUTANEOUS ABSORPTION OF METHYLENE-bis-O-CHLOROANILINE (MbOCA) AND METHYLENEDIANILINE (MDA) THROUGH RAT SKIN IN VITRO.

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Methylene-bis-o-chloroaniline (MbOCA) and methylenedianiline (MDA) are widely used industrial chemicals to which there is significant human exposure via the skin. We have evaluated the disposition of these chemicals after topical application to full-thickness Fischer 344 rat skin in flow-through diffusion cells, using a technique previously described as a model for *in vivo* percutaneous absorption (Hotchkiss et al. Fd Chem. Toxic., 28, 443-447, 1990). After application of ^{14}C-MbOCA (37.5μg/cm^2) to rat skin, the absorption through unoccluded skin and into the receptor fluid was poor, reaching 0.91±0.04% of the applied dose at 72h. When the skin was occluded with a teflon cap, the absorption of MbOCA was enhanced, reaching 1.36±0.32% at 72h. After application of ^{14}C-MDA (40.6μg/cm^2) to unoccluded rat skin, the absorption into the receptor fluid was more rapid and extensive than for MbOCA, reaching 7.68±0.99% of the applied dose at 72h. When the skin was occluded with a teflon cap, the absorption of MDA was greatly enhanced, reaching 17.01±1.10% at 72h. At the end of each experiment, the skin was swabbed with ethanol to determine any unabsorbed material, which accounted for 17-33% of the applied dose for both chemicals. The skin was then digested in methanolic sodium hydroxide to determine any residual radioactivity within the tissue, which amounted to approximately 68% and 54% for MbOCA and MDA, respectively. The total recovery of radioactivity for all experiments was always >90%. These data indicate that there is significant absorption of topically applied MbOCA and MDA into rat skin *in vitro*. Such information, taken together with comparative data from studies which are presently being conducted in human skin, may be of significance in the safety evaluation of these chemicals after topical exposure in man.

These studies were supported by a grant from the UK Health and Safety Executive.

PROTECTIVE EFFECT OF CHELATING AGENTS AGAINST LIVER INJURY INDUCED BY GALACTOSAMINE AND ENDOTOXIN IN MICE

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Tumor necrosis factor (TNF), leukotriene D_4 and reactive oxygen species have been reported to be the mediators in the pathogenesis of galactosamine-and-endotoxin-(Gal & E) induced hepatitis. In this study, liver injury was assessed by measurement of serum glutamate-pyruvate transaminase (SGPT) after coadministration of D-galactosamine (600 mg/kg) and *Salmonella enteritidis* endotoxin (20 μg/kg) in ICR mice. The SGPT level was sharply increased from 10 to 16 hr by several hundred folds as compared with control. The specific content of hepatic cytochrome P-450 was depressed initially by 15% in 4 hr and then progressively decreased by 39% in 16 hr, whereas specific activity of microsomal 7-ethoxyresorufin deethylase was also decreased by 43% in 16 hr. It is known that dexamethasone inhibits endotoxin-induced release of TNF and interleukin-1. Pretreatment of 400 μg/kg dexamethasone fully protected the animals against liver injury, but it failed to prevent the depression of cytochrome P-450. In addition, pretreatment of mice with chelating agent, 400 mg/kg DMSA (*meso*-2,3-dimercaptosuccinic acid), also protected the animals against liver injury and partially prevented the depression of specific content and activity of cytochrome P-450 by 23% and 36% respectively. Other chelating agents such as disodium edetate and DMPS (dimercaptopropane-1-sulfonic acid) also had similar protective effects. It is suggested that metal ions may play a functional role in the depression of cytochrome P-450 as a result of liver damage induced by galactosamine and endotoxin in mice.

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CHARACTERIZATION OF DOG LIVER GLUTATHIONE S-TRANSFERASES

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In the present study we have identified at least four subunits, Yd1(Mr 26k), Yd2(Mr 27k), Yd3(Mr 28 k) and Ydf(Mr 27.4k), and purified four forms of GST, Yd1Yd1, Yd2Yd2, Yd3Yd3 and YdfYdf to apparent homogeneity. Immunoblot analysis has revealed that subunits Yd1,Yd2 and Yd3 belong to pi, alpha and mu class, respectively. On the contrary Ydf had no reactivity with antibodies raised against rat GST 1-1, 2-2, 3-3, 4-4 and 7-7. Each subunit,Yd1, Yd2 Yd3 and Ydf was distinguishable by its own retention time on reverse-phase HPLC. N-Terminal amino acid sequences of dog GSTs have indicated that Yd1Yd1 and Yd3Yd3 have a high identity to pi and mu class transferases, respectively, from rat, human and mouse, while the N-terminus of Yd2Yd2 is blocked. GST Yd3Yd3, which is immunologically associated with rat transferase 3-3, had no activity towards DCNB, a specific substrate for subunit 3, suggesting it to be a new enzyme of the mu class. GST YdfYdf possessed no homology to any of three classes transferases from rat, human and mouse in the N-terminal amino acid sequences. The most significant property towards DCNB, exceeding by one order corresponding values for the known mu class GSTs. These results strongly suggest that GST Ydf Ydf is a new enzyme belonging to as yet unidentified class.

INDUCTION OF THE CYTOCHROME P450 I AND IV FAMILIES AND PEROXISOMAL PROLIFERATION IN THE LIVER OF RATS TREATED WITH BENOXAPROFEN

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The non-steroidal anti-inflammatory drug benoxaprofen (Opren) was withdrawn in 1982 following reports of fatal hepatotoxicity. In the present study intraperitoneal administration of benoxaprofen (25, 100 and 250 mg/kg) to rats, for three days, gave rise to significant increases in the hepatic O-dealkylations of ethoxyresorufin and methoxyresorufin, two substrates associated with the P450 I family, and in the 12-hydroxylation of lauric acid, a substrate associated with the P450 IV family. Immunoblot studies employing solubilised microsomes from benoxaprofen-treated rats revealed that benoxaprofen increased the hepatic apoprotein levels of P450 IA1 and A2 and of P450 IVA1. The same treatment with benoxaprofen increased the β-oxidation of palmitoyl CoA determined in liver homogenates, and immunoblot analysis showed an increase in the levels of *trans*-2-enoyl CoA hydratase bifunctional protein. It is concluded that, in the rat, benoxaprofen is a peroxisomal proliferator which selectively induces the hepatic cytochrome P450 I and IV families and these properties may be, at least partly, responsible for its hepatotoxicity.

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PERMEATION ROUTES OF FOSFOMYCIN ACROSS SMALL INTESTINAL MEMBRANE

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Fosfomycin(FOM) which is an antibiotics with phosphate residue has been known to be absorbed from gastrointestinal tract, and has been administered orally. Since it has a low molecular weight (MW. 138) and low lipophilicity, its absorption mechanism has been interested. One of us reported that it is absorbed by a phosphate transport system into brush border membrane vesicles (BBM) of rat small intestine(T. Ishizawa et al. J.Pharmacobio-Dyn. 13, 292(1990)). This corresponds to a transcellular route. But a paracellular route is assumed another possible route for FOM absorption, because it has a small molecular weight and high hydrophilicity. In this presentation, we examined the contribution of both routes for FOM absorption through not only rat small intestinal membrane but also rabbit one by voltage clamp method. As results, it was found in both animals that FOM can be absorbed through both routes though the transcellular route is predominant at the physiological membrane potential(about 2 mV). The transcellular permeability of FOM was reduced by phosphate ion, as expected from the in vitro BBM experiments. And there was a tendency that both routes are more permeable in rabbit.

CYCLOSPORINE IN RENAL TRANSPLANTATION

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135 recipients (100 male and 35 female) of cadaveric and living or non-living donor renal transplants were maintained on immunosuppressants [prednisolone 0.2mg/kg/d, azathioprine 1 mg/kg/d and cyclosporine (CyA) 3-5 mg/kg/d]. Cyclosporine plasma level was monitored regularly by FPIA method. Plasma CyA levels were correlated with their biochemical, immunological and haematological profiles. During early post transplant period (4weeks), patients were randomly kept on low 5mg/kg/d and moderate 10 mg/kg/d dose of CyA. Except slower decline in creatinine level in recipients of moderate CyA dose, graft function was not affected by starting CyA. CyA plasma level vs hematocrit (18-57%)profile gives an initial descending and a latter ascending phase. Case studies proved that post transplant complications like nephrotoxicity, hepatotoxicity, infections, diabetes mellitus, hirsutism, kaposis sarcoma etc. were recorded in patients having stipulated CyA therapeutic range (150-300 ng/ml) or even lower plasma level. Most of the patients needed antihypertensive drugs for controlling BP. 95% patient survival rate was achieved during this 1-28 month follow up period.

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IN VIVO METABOLISM OF CIMETROPIUM BROMIDE

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Cimetropium bromide has been used clinically in the treatment of irritable bowel syndrome. The *"in vivo"* metabolism of orally administered [^{14}C]-cimetropium bromide was studied in different species (i.e. dog, rabbit, rat and mouse). Due to its quaternary nature, cimetropium bromide is ionised at all physiological pH and as a consequence it was poorly absorbed from the gut and a large percentage of the drug was recovered unchanged in the faeces.

Species differences were observed, particularly in the formation of metabolites resulting from aromatic hydroxylation or cleavage of the ester linkage and their corresponding phaseII conjugates. Between 54-86% of the dose absorbed was metabolised to 4-hydroxycimetropium, scopine, scopoline, dehydroxymethylated cimetropium, N-demethylated cimetropium and two unknown metabolites.

The presence of glucuronic acid and sulphuric acid conjugates in urine and faeces were inferred and the nature of these conjugates which were different in various species, studied. Furthermore, faecal recovery of both unconjugated and conjugated metabolites suggest the involvement of enterohepatic circulation in the *"in vivo"* metabolism of cimetropium bromide.

IN VITRO METABOLISM OF SULFATHIAZOLE IN RUMEN FLUID AND ITS DISPOSITION KINETICS FOLLOWING INTRARUMINAL ADMINISTRATION IN GERMAN BLACK HEAD SHEEP

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The antimicrobial agents may undergo a change in the complex stomach particularly in rumen as a result of microbial fermentation in ruminants. The present investigation deals with the influence of ruminal microorganisms on the degradation of sulfathiazole (STZ) in in vitro and its disposition kinetics and metabolism following intraruminal administration of single dose (100 mg/kg b.wt.) in adult German black head sheep. The drug is metabolized to N^4-acetyl sulfathiazole in rumen fluid after in vitro incubation at different concentration at different time intervals at 38 ± 0.5° C. However, it is metabolized significantly to its N^4-acetyl metabolite in the rumen after intraruminal administration. The levels of STZ were maintained above therapeutic concentration (above 40 µg/ml) till 24 hours in the rumen fluid. However, the levels of STZ in plasma could not be achieved upto the minimum therapeutic concentration at any time interval after intraruminal administration. This indicates that the drug is poorly absorbed in the circulation. The biological half-life of STZ was 16.70 h and the area under the curve was 686.07 µg/ml/h.

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IN VIVO CONVERSION OF BENZO(A)PYRENE-7,8-DIHYDRODIOL TO 7-HYDROXYBENZO(A)PYRENE IN THE AMERICAN LOBSTER.

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In many species, benzo(a)pyrene-7,8-dihydrodiol (BaP7,8D) is biotransformed to the carcinogenic BaP7,8D-9,10-epoxide metabolite which can bind to DNA and initiate carcinogenesis. Competing pathways of biotransformation of BaP7,8D are conjugation with carbohydrate or sulfate. [^{14}C]-BaP7,8D was administered orally or intravascularly to groups of lobsters, which were sacrificed at 1 to 16 days after the dose. At all sampling times, hepatopancreas and muscle contained the highest percentages of dose and the antennal gland and intestinal contents had the highest concentrations of [^{14}C]. Analysis of muscle at one to eight days after the dose showed only unchanged BaP7,8D (45-73% of the [^{14}C] present), acid-hydrolysable conjugates of BaP7,8D (14-22% of [^{14}C] present) and and small amounts of unextractable residue. Hepatopancreas taken at one or two days after the dose contained unchanged BaP7,8D (20-40% of [^{14}C] present), acid-hydrolysable conjugates of BaP7,8D (2-6%) and of 7-hydroxyBaP (2-16%), small amounts of tetraols and some unextractable material. Antennal gland also contained 7-hydroxyBaP, BaP7,8D and conjugates of both 7-hydroxyBaP and BaP7,8D. The presence of conjugates of 7-hydroxyBaP in hepatopancreas and antennal gland suggests a novel pathway for biotransformation of BaP7,8D in the lobster - dehydration followed by efficient conjugation of the 7-hydroxyBaP.

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V79 CELLS GENETICALLY ENGINEERED FOR EXPRESSION OF CYTOCHROME P450IA2 AND METABOLICAL AND GENOTOXICOLOGICAL STUDIES OF AROMATIC AMINES

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In continuation of our work on the establishment of a cell bank for metabolical and genotoxicological studies, V79 Chinese hamster cells were genetically engineered for stable expression of rat cytochrome P450IA2. Cytochrome P450IA2 plays a crucial role in the biotransformation and the metabolic activation of aromatic amines.

A full-lenght P450IA2 cDNA clone was isolated from a cDNA library prepared from Aroclor 1254-treated rat liver. V79 cells were transfected with a pSV2-gpt derived recombinant eukaryotic expression vector containing the P450IA2 cDNA. Two transfected cell lines, XEMd-MZ and XEMd-NH, stably expressing P450IA2 protein were obtained. XEMd-NH cells were derived from a V79 variant line that constitutively expressed the phase II enzyme N-acetyl-transferase. The transfected cell lines were checked for chromosomal integration of the cDNA by Southern-blotting, for expression of an authentic mRNA and protein by Northern- and Western-blotting.

V79 cell lines expressing P450IA2 after transfection were enzymatically active as demonstrated by the hydroxylation of 2-aminofluorene and 17ß-estradiol. In addition, XEMd-MZ cells were shown to be capable of specific demethylation of methylxanthines like caffeine, which may be used as indicator drug in pharmacological studies (in collaboration with U.Fuhr and A.H.Staib, Klinische Pharmakologie, Frankfurt a.M.).

Mutagenicity of several aromatic amines like 2-aminoanthracene, 2-aminofluorene and 6-aminochrysene was only observed in XEMd-NH cells indicating that both enzymes, P450IA2 and N-acetyl-transferase were needed to create an ultimate mutagen.

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THE RESPONSIBILITY OF CYTOCHROME P450 FOR HYDRAZINE TOXICITY *IN VIVO*.

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The metabolism and biochemical mechanisms of toxicity for hydrazine have not yet been fully defined. Previous *in vitro* studies have shown that hydrazine is oxidatively metabolised in microsomes by cytochrome P450 [1]. Hydrazine hepatotoxicity is manifested as steatosis, accompanied by depletion of ATP and glutathione (GSH) and in this study the dose response relationship of hydrazine (0-60 mg/kg) for the 3 indices of toxicity was established for male Sprague Dawley rats (250-300g), 6 hours after an i.p. dose. Pretreatment of animals with various inhibitors and inducers of cytochrome P450 produced a shift in the dose response curve and these shifts were found to be significant after the effect of pretreatment over non-pretreatment had been eliminated from the data. Pretreatment with the inhibitor piperonyl butoxide resulted in an increase in toxicity, while induction by phenobarbitone (P450IIB) or β-Napthoflavone (P450IA) decreased toxicity. In contrast acetone (P450IIE) induction caused an increase in toxicity.

The results demonstrate that hepatotoxicity of hydrazine can be modulated by inducing and inhibiting various forms of cytochrome P450 and suggest that individual isoenzymes may be implicated in the metabolism of hydrazine by different processes.

This is of particular interest in view of recent studies which have shown that hydrazine itself alters the activity of certain cytochrome P450 subfamilies.

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FORMATION OF AFLATOXIN B_1-DNA ADDUCTS IN VIVO IN RAT LIVER SINUSOIDAL CELLS.

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The liver of the adult rat is composed of around 65% parenchymal cells (PC) and 35% non-parenchymal, mainly Kupffer and endothelial, cells (NPC). Rats administered aflatoxin B_1 (AFB_1) develop PC-derived hepatocellular carcinomas but no tumours derived from Kupffer or endothelial cells have been reported. The number of tumours induced correlates with the levels of DNA-adducts formed in the livers of AFB_1-treated rats. In the present study the in vivo formation of AFB_1-DNA adducts within PC and NPC populations was analysed.

PC and NPC fractions were isolated from the livers of male Sprague-Dawley rats by in situ perfusion via the portal vein with collagenase H and pronase E followed by density gradient centrifugation in Nycodenz. Animals were injected intraperitoneally at 12-24 hours before perfusion, with tritiated AFB_1. Levels of AFB_1-DNA adducts were determined by HPLC and scintillation counting. Significantly higher adduct levels were found in the DNA from PC than from NPC fractions. In both cell types DNA-bound radioactivity coeluted with a standard for the guanyl imidazole ring-opened adduct of AFB_1. Isolated cells contained substantially lower DNA-adduct levels than were reported for fresh whole livers from AFB_1-treated rats. This was confirmed using male Sprague-Dawley and Fischer F344 rats given AFB_1 one hour prior to liver excision. Interestingly, Fischer F344 rats produced 4-6 times more DNA-bound AFB_1 as did Sprague-Dawley rats.

The decline in adduct levels during cell isolation was not due to non-specific removal by impurities in the collagenase or pronase preparations as these enzymes failed to liberate any radioactivity from tritiated AFB_1-modified calf-thymus DNA in in vitro incubations.

In conclusion, this study shows that AFB_1 is activated in vivo in the rat liver to form DNA-adducts in NPC but at lower levels than in PC.

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FLUCONAZOLE : METABOLIC STABILITY AND RENAL CLEARANCE LEAD TO PREDICTABILITY OF PHARMACOKINETICS IN MAN.

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Fluconazole (Diflucan) is an antifungal drug which is essentially stable to metabolism and mostly eliminated via the kidney in all species, including man. Allometric comparison of renal clearances (Cl_R) and glomerular filtration rates (GFR) across the species indicates that these two processes are intimately associated. This has been confirmed for dog and man where the dependence of CL_R on GFR has been established. No active processess such as secretion are believed to be involved. In addition, experiments in the dog have shown that a constant 80% of fluconazole is reabsorbed from the tubule, irrespective of variations in GFR. A possible explanation is that the drug is reabsorbed, with most of the water, in the proximal tubule, with negligible reabsorption from the distal tubule. This is in keeping with the observation that coadministration of fluconazole with common diuretics (which act on the distal tubule) does not lead to an increase in clearance. In conclusion, the pharmacokinetic properties of Fluconazole are largely predictable on the basis of renal function and unlikely to be affected by concomitant administration of drugs.

METABOLIC STUDIES WITH [^{125}I]-DEFIBROTIDE: METHODS FOR DETERMINATION OF THE PROPORTION OF FREE IODIDE IN BIOLOGICAL SAMPLES

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Defibrotide is an orally active antithrombotic and fibrinolytic agent composed of polydeoxyribonucleotides of molecular weight between 15,000 and 30,000 daltons. The blood kinetics of total radioactivity following oral and intravenous administration of [^{125}I]-defibrotide to rats has been examined. Defibrotide was radiolabelled by the Iodo-gen method and the biological activity of the radiolabelled material was checked before use. A comparison of data from kinetic studies with Na^{125}I and [^{125}I]-defibrotide suggested that a significant proportion of total radioactivity detected in blood of rats following oral administration of [^{125}I]-defibrotide may be present as free radiolabelled iodide. In order to define the pharmacokinetic parameters of defibrotide related components a simple method to distinguish between free iodide and iodide associated with defibrotide components was required. We have examined a variety of methods to distinguish between free radioactive iodide and radioactive iodide associated with defibrotide and defibrotide related components in biological samples in vitro and ex vivo. Methods examined included ultrafiltration and precipitation techniques, chromatographic methods (TLC and simple column chromatography) and electrophoresis. With the exception of the precipitation technique all of these methods were shown to be capable of quantitatively determining the proportion of radioactivity present in biological samples as free iodide. A simple column chromatographic method was selected for routine use on the basis of its simplicity and good sample capacity as well as its ability to resolve likely small molecular weight products of [^{125}I]-defibrotide from free iodide. The determination of the proportion of free iodide in samples ex vivo does not preclude the need to examine the nature of circulating breakdown products of the polydeoxyribonucleotides in terms of molecular weight and chromatographic character. Further such studies are now in progress.

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MOLECULAR CLONING AND EXPRESSIONS OF POLYMORPHIC ACETYL-TRANSFERASE OF HAMSTER LIVER

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We have recently purified two forms of acetyltransferase, AT-I and AT-II, which show the difference in molecular weight and catalytic activity in N-acetylation of arylamines and O-acetylation of N-hydroxyarylamines (Carcinogenesis, 11, 1512, 1989). Here, we report molecular cloning, a complete nucleotide sequences of AT-I and expression in monkey kidney COS-1 cells and E. Coli to identify the molecular species and to characterize more precisely the catalytic property of AT-I. The cloned cDNA, ham AT 101, consisted of 1181 nucleotides, with a region of 870 nucleotides coding for 290 amino acids (MW 33,503) included. In Northern blots, the isolated cDNA probe hybridized with a single band at 18s of hamster liver RNA in both slow and rapid acetylators. AT-I expressed in COS-1 cells and E. Coli showed substrate specificity similar to AT-I purified from hamster livers, but the substrate specificity was completely different from that of AT-II. Both expressed enzymes showed high capacity for the N-acetylation of 4-aminoazobenzene and 2-aminofluorene, and the O-acetylation of N-hydroxy-Glu-P-1(2-hydroxyamino-6-methyldipyrido 1,2-a:3',2'-d imidazole), whereas no activity was found for the N-acetylation of p-aminobenzoic acid. The expressed protein also capable of using N-hydroxyaryl-acetamide as an acetyl donor in contrast to AT-II purified from hamster livers which is capable of using only acetyl Co-A as acetyl donor. The results indicate that ham AT 101 is a cDNA encoding a hamster acetyltrans-ferase, AT-I. In conclusion, the deficiency of AT-II is considered to be a cause of acetylation polymorphism in hamsters.

EFFECT OF ERYTHROCYTE UPTAKE ON THE CYCLOSPORIN(CyA) KINETICS

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The relation between the erythrocyte(RBC) uptake process and the pharmacokinetics of CyA was evaluated. We measured both the specific binding and the membrane transport parameters by in vitro techniques. The parameter estimates were applied to a physiologic model analysis and the effect of RBC binding on the in vivo CyA disposition was examined in rats.

Two sets of in vitro experiment were performed using the blood from male Wistar rats; 1)a static incubation with various concentration of ^{3}H-CyA (10 min, 37°C) and 2) a kinetic measurement of tracer ^{3}H-CyA uptake. The first data set was fitted to a single binding site model: binding capacity (nPt= 3680nM) and affinity (K_d=149nM) in rats were similar to those reported for human. The second set was fitted to a linear kinetic model and the plasma-RBC uptake rate constant (0.015 ± 0.007sec^{-1}; mean ± SD) was estimated; the half-time of this uptake process (50 sec) was longer than the transit time of blood in many organs (e.g., 9 sec for liver).

In vivo disposition of CyA was simulated by adopting a physiologic model with two extreme assumptions: plasma-RBC exchange happens 1)instantaneously or 2)with the rates measured above only in the large vessels and not during the tissue capillary transit of blood. Blood concentrations after i.v. administration was higher in case-2) than in case-1) under linear condition. The largest difference (60 %) was observed during the first minute. When the RBC binding site is saturated, the difference between the two cases was negligible. These results suggest that the capacity of the RBC binding as well as the rate of plasma-RBC transport are important factors to predict the blood CyA levels in vivo.

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ROLE OF P450 3A4 IN 10,11-EPOXIDATION OF CARBAMAZEPINE

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Cytochrome P450-dependent 10,11-epoxidation is the primary route of elimination for the anticonvulsant drug carbamazepine (CBZ), in humans. Although the identity of the P450 form(s) responsible for the catalysis of this reaction have not been elucidated to date, reports of CBZ drug interactions with the P450 inducers phenobarbital and phenytoin and the P450 inhibitors erythromycin and triacetyloleandomycin (TAO) suggest the involvement of a member of the 3A subfamily. Therefore, we sought to determine the role of P450 3A4, a major constitutive P450 in human liver, in the metabolism of CBZ to CBZ-10,11-epoxide (CBZE).

P450 3A4 was purified from liver tissue obtained from a 13yr old organ donor. The identity of the purified enzyme was made on the basis of its N-terminal amino acid sequence and its regiospecific catalytic activity towards the substrates testosterone and midazolam. Polyclonal antibody was raised against the enzyme and it was found by Western blot to recognize microsomal P450 3A4 (and probably 3A3), and in approximately 15% of livers examined, a protein that is most likely P450 HLp3. Reconstituted P450 3A4 catalyzed the formation of CBZE (20-250 pmol/nmol P450/min) from CBZ (15-180μM); the reaction velocity appeared linear across this concentration range. Enzyme kinetic constants could not be determined because of substrate insolubility at higher concentrations. Inclusion of the P450 3A inhibitor TAO in incubations with human liver microsomes decreased the rate of CBZE formation from CBZ (100μM) to 23% of control (maximal inhibition). Polyclonal anti-3A4 IgG also inhibited the formation of CBZE in incubations with human liver microsomes, in a concentration dependent manner. The reaction velocity was reduced to 36% of control at an IgG concentration of 15mg/nmol P450.

These results provide preliminary evidence that P450 3A4 is an important catalyst of CBZ clearance in humans, and that induction or inhibition of this enzyme is a likely mechanism underlying *in vivo* drug interactions in which CBZ clearance is altered.

CULTURE CONDITIONS FOR RUMINANT HEPATOCYTES IN DRUG METABOLISM STUDIES.

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Introduction Culture media have generally been designed for hepatocytes derived from monogastric animal species, particularly rodents. For these species, compared to ruminants, striking differences in composition of portal blood are observed, especially with respect to glucose and volatile fatty acid (VFA) concentrations. In short term cultures (8 hr) of bovine hepatocytes, Shull *et al.* [1] reporred a rapid decrease in drug metabo-lizing capacity. These authors suggest a correlation with composition of the medium and attachment substratum. The aim of the present study was to investigate the effects of modifying Williams' Medium E and of collagen or fibronectin coating on goat hepatocyte culture performance. Modifications included the addition of a mixture of VFA and/or the reduction of glucose concentration, thus more closely approaching the *in vivo* environment of the ruminant liver.

Methods and materials Livers were removed surgically from three healthy castrated male dwarf goats (aged five, 45-50 kg). The quadrate lobe (30 g) was cut off and its blood vessels flushed with icecold saline. Cell were isolated using a three step perfusion technique. Hepatocytes were cultured in Williams' Medium E (WME) or Half Glucose WME (HGWE), containing 1.2 g glucose per liter. Both media were supplemented with 5% serum (NCS). Four series of cultures were prepared (series 1,2: WME; 3,4: HGWE). WME and HGWE of series 2 and 4 was supplemented with a mixture of volatile fatty acids (VFA): 1.5 mM propionate, 1 mM acetate, 1 mM DL-lactate and 0.5 mM D-3-hydroxy butyrate (final concentrations). In each series, cells were cultured in non-coated, calf skin collagen (CSC) and fibronectin-coated (FBN) dishes. After 3 hours preincubation, the medium was refreshed. At 24 hours, the culture medium was sampled for the spectofoto-metric determination of phenol red (PhR) glucuronidation and replaced with NCS free medium. Scoparone (6,7-dimethoxy coumarin) and ethylmorphine were incubated for two hours. Metabolites were determined using HPLC in deglucuronized medium samples. Cytochrome P450 and protein were measured at 0, 3, 27 and 51 hr.

Results and conclusions A quick onset of cell attachment and stretching was observed, especially in CSC and FBN-coated dishes. In all cultures, monolayers of cells were formed within 27 h. After an initial drop (10-20% within 3 hr) in culture P450 content, only a slow decrease in P450 level was observed from 3 to 51 hr. No influence of medium composition or dish coating was observed. The presence of VFA caused a larger initial P450 loss (20% vs. 10%), but within 27 hr P450 content was equal to non-VFA supplemented cultures (approximately 75% of 0 hr P450). Neither the biotransformation of scoparone nor that of ethylmorphine was influenced by the culture conditions investigated. The glucuronidation of PhR did not yield significant differences. Addition of VFA to WME or HGWE tends to decrease P450-mediated metabolism but does not unambiguously affect PhR glucuronidation.

With all combinations, culture quality was high for at least 51 hr. The presence of VFA seems to delay the cell recovery from the "perfusion-stress", but this is only temporarily. We therefore conclude that WME is a suitable basal medium for primary cultures of goat hepatocytes used in drug metabolism studies. Supplementing with the VFA studied nor coating of dishes with CSC or FBN adds to the culture performance.

Ref. 1. Shull,L.R., Kirsch,D.G., Lohse,C.L. *et al.*, Am.J.Vet.Res. 47 (1986) 2043.

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BIOSYNTHESIS OF TOXIC GLUTATHIONE S-CONJUGATES: THEIR POSSIBLE ROLE IN AMINOPHENOL-NEPHROTOXICITY

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p-Aminophenol is a renal proximal tubule toxicant in the rat. After 100 mg/kg aminophenol i.p., almost 70 % of dose is recovered as biliary metabolites within 5 h. The major metabolite was an acetaminophen conjugate; 4-amino-3-glutathion-S-yl-phenol and 4-amino-2-glutathion-S-yl-phenol were also present (3 % of dose). Both these glutathione S-conjugates and unchanged p-aminophenol may be responsible for nephrotoxicity due to active accumulation. To obtain information on the uptake of these glutathione S-conjugates and aminophenol, we studied their cytotoxicity in rat kidney cortex cells in the presence of agents modifying renal uptake mechanisms. Aminophenol glutathione S-conjugates were toxic to rat kidney cells, their toxicity is dependent on accumulation by a γ-glutamyl transpeptidase-dependent system. Aminophenol also induced time- and concentration-dependent cell killing; inhibition of cytochrome P-450 and the presence of tetraethylammonium bromide substantially decreased p-aminophenol cytotoxicity. The obtained results suggest that p-aminophenol is accumulated in kidney cells by a cation transporter. In the kidney *in vivo*, this transporter is mainly situated in the S-1 segment, which is not the target for aminophenol nephrotoxicity. Glutathione conjugates may therefore be responsible for p-aminophenol nephrotoxicity *in vivo*.

THE ROLE OF LONG CHAIN FATTY ACID CoA LIGASE IN THE METABOLISM OF XENOBIOTIC CARBOXYLIC ACIDS

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Hepatic coenzyme A ligases catalyzing the formation of acyl-CoA thioesters of fatty acids and xenobiotic carboxylic acids can be divided into three types, i.e. short, medium and long chain fatty acid CoA ligases (E.C.6.2.1.1-2.1.3). Of these, the mitochondrial medium chain is principally associated with activation of benzoic and phenylacetic acids while the microsomal long chain ligase (LCL) has been implicated in the stereospecific formation of the CoA ester of the R enantiomers of fenoprofen and ibuprofen. LCL is also located in the outer peroxisomal membrane and a variety of carboxylic acids which induce peroxisomal proliferation are activated to CoA intermediates. To date there is no evidence concerning the role of the peroxisomal ligase in this reaction. Peroxisomes were isolated from rodent liver and endogenous ligase activity determined using ^{14}C-palmitate. Two forms of the enzyme were demonstrated,one exhibiting a high (K_m 3.35 μM) and low affinity (K_m>1.2mM) for palmitic acid. The R & S enantiomers of ibuprofen and naproxen non-competitively inhibited the high affinity form while minimal effect was observed on the low affinity form of the enzyme. In contrast, clofibric acid, valproic acid, 2,4-D and salicylic acid did not inhibit either form of the enzyme. These data are in contrast to that reported using the microsomal LCL which has been shown to catalyze R-fenoprofen and clofibroyl-CoA formation, thus suggesting that these two long chain ligases differ in xenobiotic substrate specificity.

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